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A Comparison of the Proteins of Normal and Trichothiodystrophic Human Hair

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A study has been made of the changes in the constituent proteins of a sample of sulfur-deficient human hair (trichothiodystrophy, TTD) which has a cystine content less than 50% of normal. The constituent low-sulfur proteins, although increased in amount, appear normal (apart from the absence of one minor component) as they contain the same array of subunit polypeptides covering the same range of molecular weights and isoelectric points as normal hair. In contrast, the high-sulfur proteins not only were decreased from the normal 40–45% to less than 10% but the proteins were on average smaller, had a lower than normal cystine content, and had a greatly altered amino acid composition. Fractionation followed by two-dimensional electrophoresis of the fractions revealed that the TTD high-sulfur proteins had lost the large heterogeneous group of ultrahigh-sulfur proteins and at least 8 major high-sulfur protein components but had acquired at least 22 components of lower than normal cystine content, which are either not present in normal human hair or are present at levels too low for detection. It is suggested, on the basis of nutritional studies on sheep and humans, that the loss of the ultrahigh-sulfur proteins is due to the presence of a sulfur-deficiency state, presumably of metabolic origin. The changes in the other high-sulfur proteins may be due to the mutation having affected the operation of a regulatory gene for high-sulfur proteins, as such changes do not occur in wool or hair as a result of dietary manipulation.

There have been a number of reports, reviewed by Price [1] and Goldsmith [2], of the occurrence of brittle human hair which, compared with normal hair, is abnormally low in cystine content. This syndrome, now termed trichothiodystrophy (TTD) [1] is often, but not invariably, accompanied by a range of other disorders including skin and nail abnormalities. An autosomal recessive inheritance pattern has been found by one group of workers [3]. There is a general concensus that the decreased cystine content of the hair is due both to a reduction in the proportion of high-sulfur proteins and to a decreased synthesis of those components richest in sulfur [2, 4–9]. There is, however, some disagreement over the existence of changes in the low-sulfur proteins [6,9]. The actual mechanism by which the presumptive mutation affects the synthesis of the hair proteins has not been elucidated, but on the basis of analogy with the changes in wool proteins caused by sulfur deprivation, i.e., a decrease in total high-sulfur protein and a concurrent decrease in their cystine content, and with the changes in hair proteins associated with kwashiorkor, various workers have suggested that the mutation causes a metabolic defect resulting in a deficiency in the availability of sulfur-containing amino acids for hair synthesis and presumably for other protein synthesizing systems as well [5, 6]. Price et al [10] list a large number of other syndromes which to varying degrees are associated with TTD, suggesting that we are observing the effects of not one but a number of inherited defects which may have, as suggested by Brown et al [5], different mechanisms but a common end result in a deficiency in the synthesis of hair high-sulfur protein.

A number of questions were left unanswered or only partly answered by previous workers, and the availability of a cystine-deficient hair has provided the opportunity for us to use some new high-resolution electrophoretic techniques that have become available since the very thorough study of Pollitt and Stonier [6]. We were interested in determining whether the inherited defect changed not only the amount but also the nature of the high-sulfur proteins, resulting in the appearance of new high-sulfur protein components. If TTD comprises a group of inherited disorders all resulting in a changed pattern of synthesis of the high-sulfur proteins then we might expect to find quantitative differences in the changes in the proteins of hair from different affected individuals. It is of considerable interest to know whether the low sulfur proteins are affected by the disorder because it would be surprising indeed if the microfibril could tolerate, without serious disruption, significant changes to the amino acid sequences of the subunit polypeptide chains or their relative proportions.

When Brown and his coworkers [5] and Pollitt and Stonier [6] explained the changes in TTD hair as the result of a metabolic sulfur deficiency they relied on results from this laboratory on diet-induced changes in the proteins of wool and hair. In order to see whether further light can be shed on this problem and to set the interpretation on a sounder basis we have reexamined the changes in hair that occur during kwashiorkor and in wool during periods of sulfur deprivation and sulfur enrichment.

MATERIALS AND METHODS

Preparation of Soluble Hair Proteins

The TTD hair was the gift of Dr. V. H. Price. Its origin and characteristics are described in [10] where it is identified as sample 1. Unfortunately the comparatively small size of the sample coupled with the low content of high-sulfur proteins greatly restricted the scope of our studies on these proteins. The control was provided by a sample of normal hair from a 2-year-old boy, the son of one of us (RCM). Both samples were unusually soluble (cf [9]) (~ 75%) in the urea-mercaptoethanol extractant. The origin of the other hair and wool samples is given in the text.

The various samples of human hair and wool were washed successively with petroleum ether, ethanol, and water and then air dried. They were solubilized by treatment with 0.2 M β-mercaptoethanol in 8 M urea at pH 11 for 2 h and the cysteinyl residues of the soluble proteins stabilized by alkylation with iodoacetate to convert them to S-carboxymethylcysteinyl (SCMC) residues. After dialysis, the constituent proteins were separated by adding zinc acetate to 0.02 M giving a precipitate of low-sulfur protein and leaving the high-sulfur proteins in the supernatant. Both protein fractions were treated with excess sodium citrate to remove the zinc ions, dialyzed, and freeze-dried. For further details consult [11]. In one experiment the proteins were extracted with 8 M urea–0.05 M dithiothreitol–0.05 M Tris at pH 9.3 for 18 h at room

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Abbreviations:
HPLC: high performance liquid chromatography
PAA: polyacrylamide
SCMC: S-carboxymethylcysteinyl
SDS: sodium dodecyl sulfate
TTD: trichothiodystrophy
UHS: ultrahigh sulfur

temperature, partly alkylated with iodo[2-^{14}C]-acetic acid and then fully alkylated with excess unlabeled iodoacetic acid [12].

Chromatography of High-Sulfur Proteins

The freeze-dried samples of high-sulfur proteins were dissolved in the buffer system 0.1 M ammonium bicarbonate—8 M urea–0.1 M sodium chloride, sucrose added to 20% w/v, and the solutions applied to a 1.6 × 90 cm column of Sephadex G-200 equilibrated with buffer. The proteins were eluted using a flow rate of about 5 ml/h. Five milliliter fractions were collected, pooled as indicated on the profiles (see Fig. 3), dialyzed against deionized water, and freeze-dried.

Amino Acid Analysis

Hair proteins were hydrolyzed in vacuo with constant boiling HCl at 108°C for 22 h, freeze-dried, and the content of amino acids estimated with a modified Beckman 120C amino acid analyzer. Hair was treated in a similar way but after freeze-drying, the hydrolysate was adjusted to pH 7–8 and shaken with air to oxidize cysteine.

Electrophoresis

The low-sulfur protein subunit polypeptides were separated by two-dimensional electrophoresis using in the first dimension either isoelectric focusing or electrophoresis in polyacrylamide (PAA) gel rods at pH 8.9 in the presence of 8 M urea, followed by electrophoresis in PAA gel slabs at pH 8.9 in the presence of sodium dodecyl sulfate (SDS).

One-dimensional electrophoresis of the high-sulfur proteins was performed in 10% PAA gel slabs containing 4.8 M acetic acid and 2.8 M urea at pH 2.6 for 2 h at 300 V. For two-dimensional electrophoresis, the proteins were first electrophoresed in PAA gel rods (100 × 3.5 mm) using this buffer system, followed by electrophoresis in continuous gradient PAA slab gels (Gradipore) in the presence of SDS at pH 7. At the completion of electrophoresis, unless otherwise stated, proteins were located in the gels by staining with Coomassie Brilliant Blue G250. Further experimental details for the electrophoretic techniques are given in [13] and [14].

Chromatography by High Performance Liquid Chromatography (HPLC)

The M_r distribution among high-sulfur components was studied by chromatography in 0.1 M phosphate–8 M urea, pH 7 on 2 Waters I-250 columns in series (7.8 × 300 mm) using a Waters Associates HPLC system. The columns were calibrated with 3 S-carboxymethylated wool high-sulfur proteins (B2A, IIIA1 and IIIB2) of known sequence M_rs [15] and the assumption was made that the human hair high-sulfur proteins have the same elution volume–M_r relationships.

RESULTS

A Comparison of the Amino Acid and Protein Composition of TTD and Normal Human Hair

This sample of TTD hair as compared to normal hair from a 2-year-old child (Table I) had a strikingly different amino acid composition containing much less cystine, proline, serine, and threonine but substantially more lysine, aspartic acid, glutamic acid, alanine, leucine, isoleucine, and phenylalanine. From the known compositions of the constituent proteins these differences in composition are consistent with TTD hair containing much less high-sulfur protein and consequently more low-sulfur protein than normal. Isolation and gravimetric quantitation confirmed this conclusion, showing that the high-sulfur proteins had declined from the normal 40–45% to less than 10% with an equivalent increase in the low-sulfur proteins.

We next examined the constituent proteins in some detail looking for changes not only in the proportions but in the composition of the subunit polypeptides.

A Comparison of the Proteins of TTD and Normal Hair

The low-sulfur proteins: Low-sulfur proteins isolated from normal and TTD hair were compared by two-dimensional electrophoresis using isoelectric focusing in the first dimension and SDS electrophoresis in the second. It can be seen (Fig 1*a*, *c*) that each preparation contains the same number of major components in about the same relative proportions with the isoelectric points and apparent M_rs of corresponding subunit polypeptides the same. A second comparison (Fig 1*b*, *d*) using electrophoresis in 8 M urea at pH 8.9 in the first dimension, followed by SDS electrophoresis in the second confirmed the apparent identity of the major constituents but suggested some variation in relative proportions. The mutation therefore does not appear to have altered the size, isoelectric points, or net charge at pH 8.9 of the constituent low-sulfur polypeptide chains. Therefore it was surprising to find that the TTD low-sulfur protein contained about 26% less SCMC than the normal material (Table I), a difference in composition which should be reflected in a set of higher than normal isoelectric points. We have evidence that this difference in composition is due to the absence in TTD hair of a particular high-sulfur protein component which normally contaminates preparations of the low-sulfur proteins of human hair and gives them an anomalously high SCMC content. It is possible that the band of low isoelectric point protein (arrow in top left-hand corner, Fig 1*a*) represents this contaminating protein.

TABLE I. *Amino acid compositions (as residues %) of normal hair, TTD hair, and their constituent proteins*

Amino acid	Normal hair			TTD hair		
	Hair	Low-sulfur proteins	High-sulfur proteins	Hair	Low-sulfur proteins	High-sulfur proteins
Lysine	2.7	3.5	0.5	3.6	3.9	1.2
Histidine	0.9	0.7	0.8	0.9	0.8	1.1
Arginine	5.8	7.1	6.2	5.7	6.8	5.2
SCMC[a]	NP[b]	7.6	27.2	NP	5.6	18.6
Aspartic acid	4.9	9.3	2.2	8.3	9.3	3.3
Threonine	6.8	5.4	11.2	5.4	4.8	9.0
Serine	11.7	8.9	13.1	9.5	8.5	13.5
Glutamic acid	11.4	16.5	8.0	14.2	16.8	8.1
Proline	8.4	3.8	12.0	5.7	4.2	11.5
Glycine	6.4	5.1	5.5	6.7	5.9	8.4
Alanine	4.6	6.9	1.9	7.1	7.1	3.6
½ Cystine[c]	17.8	NP	NP	8.0	NP	NP
Valine	5.8	6.1	5.2	6.2	5.8	6.1
Methionine	0.6	0.4	0.0	0.9	0.8	0.2
Isoleucine	2.6	3.6	1.4	3.5	4.0	2.4
Leucine	5.8	10.2	2.2	9.1	10.7	4.0
Tyrosine	2.0	2.5	1.5	2.7	2.9	1.9
Phenylalanine	1.6	1.9	1.1	2.4	2.3	2.0

[a] S-carboxymethylcysteine
[b] NP – not present.
[c] Estimated as cysteic acid

These electrophoretic comparisons also revealed that TTD hair lacks a minor low-sulfur protein (less than 10%) which was present in the control hair (marked with an arrow in Fig 1*a*, *b*) and also 7 other hair and nail samples examined by this technique. It may well be that the loss of this component is the result of the TTD mutation; however, without examining hair from other members of the subject's family we cannot rule out natural polymorphism as the cause.

The high-sulfur proteins: A comparison of normal and TTD high-sulfur proteins by electrophoresis in one dimension at pH 2.6 (Fig 2*a*, *b*) reveals marked differences between them, the pattern of the TTD protein lacks most of the smear of low-mobility proteins which are such a conspicuous feature of the normal pattern, but has 6 well-defined bands, few of which are present in the normal pattern.

The remarkably different patterns of spots given when the two proteins are examined by two-dimensional electrophoresis (Fig 2*c*,*d*) support the findings from one-dimensional electrophoresis, further suggesting that the two preparations share few common constituents. Further support for this concept will be presented later in a discussion of the fractionation studies. The most obvious difference between the two patterns is the absence from the TTD fraction of most of the high M_r proteins which form an unresolved wedge of stain in the top left-hand side of the gel. These components, termed ultrahigh-sulfur (UHS) proteins [16] are extremely rich in cystine and their

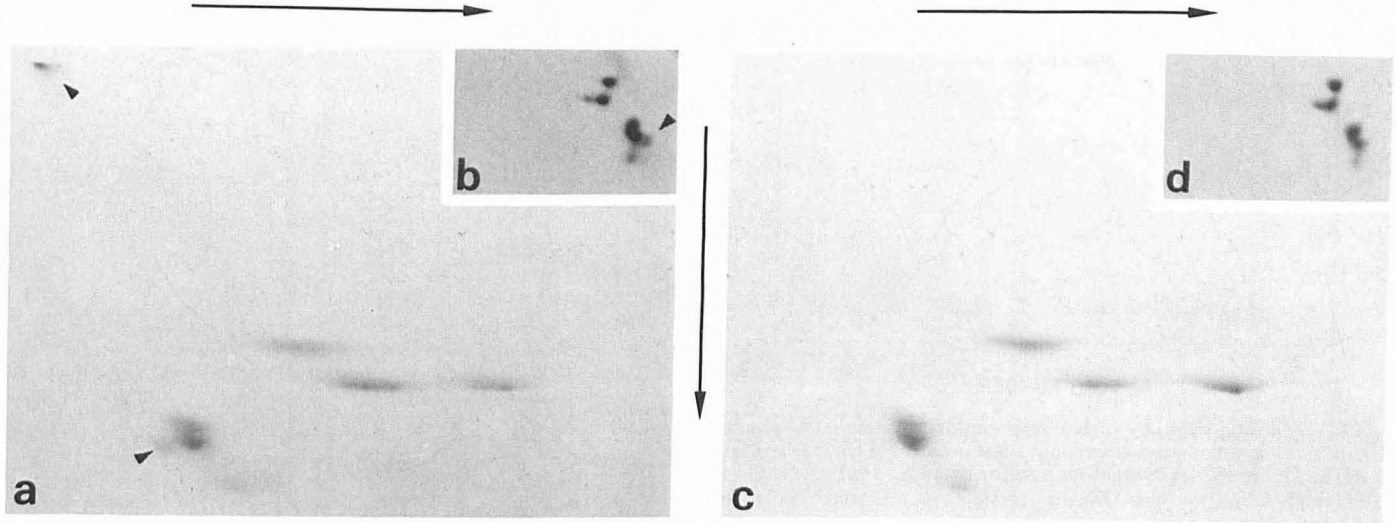

FIG 1. Two-dimensional electrophoresis patterns of the low-sulfur proteins of normal (*a,b*) and TTD (*c,d*) hairs. *a and c,* First dimension (horizontal), isoelectric focusing in 5% PAA gels containing 8 M urea. Second dimension (vertical), electrophoresis in 10% PAA gels containing SDS at pH 8.9. *b and d,* First dimension (horizontal), electrophoresis in 7.5% PAA gels containing 8 M urea at pH 8.9. Second dimension (vertical) electrophoresis in 10% PAA gels containing SDS at pH 8.9, these proteins contained S[^{14}C]M cysteine and were located by fluorography.

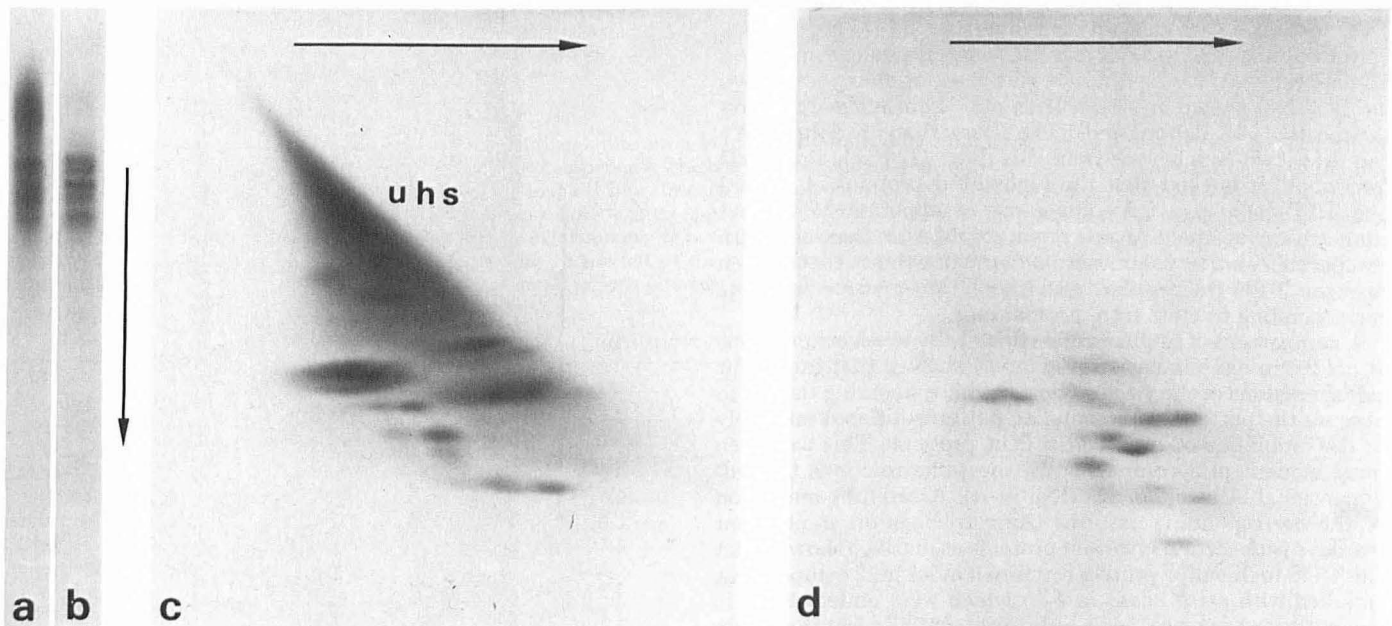

FIG 2. Polyacrylamide gel electrophoretic patterns of high-sulfur proteins isolated from normal (*a,c*) and TTD (*b,d*) hair. *a and b,* Single-dimensional electrophoresis run in 10% PAA gels containing 3 M urea at pH 2.6. *c and d,* Two-dimensional electrophoresis. First dimension (horizontal), electrophoresis in 10% PAA gels containing 3 M urea at pH 2.6. Second dimension (vertical), electrophoresis in continuous gradient PAA gels containing SDS at pH 7.

absence accounts, at least in part, for the decreased cystine content of TTD hair (Table I). There are also differences in the other components, the TTD material being richest in components with a high SDS mobility and thus lower apparent M_r, and in components of high charge and small size which run on the lower right-hand side of the gel. In line with this, ultracentrifuge studies carried out in 8 M urea buffers found that TTD high-sulfur proteins have a lower average M_r of 14,000 as compared with an average value for the normal proteins of 20,000 [7]. Further evidence for significant differences between normal and TTD high-sulfur proteins comes from an examination of their amino acid compositions (Table I). As compared with the normal, the TTD high-sulfur proteins not only contain 32% less SCMC but very much more (> 30%) aspartic acid, glycine, alanine, isoleucine, leucine, and phenylalanine. These changes in composition are consistent with the loss of the group of UHS proteins [16].

Normal and TTD high-sulfur proteins were fractionated, essentially on a size basis, by chromatography on Sephadex G-200 (Fig 3). The two elution curves although spanning the same M_r range differ markedly in shape, indicating that the two high-sulfur protein preparations contain a different size distribution, with the normal proteins being the richer in the larger molecular species and the TTD protein the richer in the smaller molecules. The eluted proteins were divided into 5 exactly corresponding fractions, labeled I–V in order of increasing elution volume and decreasing size. As expected there were marked differences between the two high-sulfur protein preparations in the yields of proteins in the 5 fractions (Fig 3), the TTD preparation for example contained a much lower proportion of the high M_r

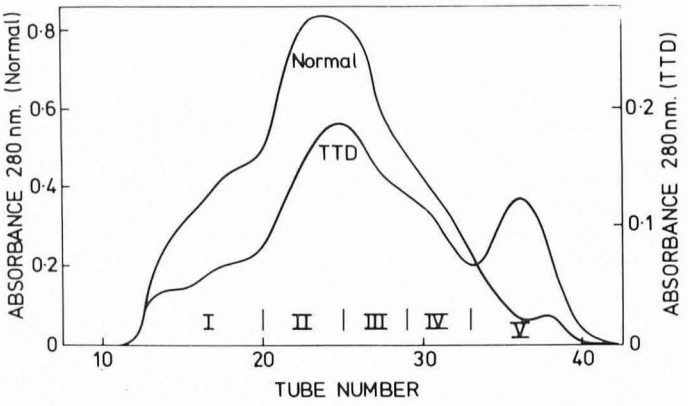

FIG 3. Chromatography of hair high-sulfur proteins on Sephadex G-200 in 0.1 M ammonium bicarbonate–8 M urea–0.1 M NaCl. Flow rate 5 ml/h. *Top profile* = normal high-sulfur proteins. *Bottom profile* = TTD high-sulfur proteins. Eluant fractions (5 ml) were pooled as indicated.

components of fraction I (15% vs. 24%) but a much larger proportion of the low M_r components of fraction V (21% vs. 5%), assuming comparable extinction coefficients. As we were unable to recover protein corresponding to normal peak V (this may not be due to protein) and only very small amounts of TTD fraction IV, of necessity our comparisons are incomplete.

Normal and TTD fractions I–III were chromatographed on M_r calibrated HPLC columns to give the elution curves shown in Fig 4. Not shown are the elution curves for normal fraction IV (mostly 6–7K dalton) and TTD V (less than 5K dalton). We can draw two conclusions from this data: (1) Excluding TTD fraction V, it is clear that the high-sulfur proteins of normal and TTD hair span the same range in apparent M_rs. (2) A comparision of the average apparent M_rs of fractions with comparable elution volumes from Sephadex shows that in each case the TTD fraction has a markedly lower value than the corresponding fraction from normal hair.

A comparison of Sephadex fractions I–IV by electrophoresis at pH 2.6 in one dimension (Fig 5*a–h*) showed that except for certain regions of the two fractions I where smearing tended to obscure the pattern, corresponding patterns differed markedly in the mobilities of their constituent proteins. This was even more evident in a comparison of the same fractions by two-dimensional electrophoresis (Fig 6*a–h*). A careful comparison of the corresponding patterns using tracings on transparent overlays with certain invariant proteins as markers showed that the TTD high-sulfur protein contained at least 22 components, (marked with arrowheads in *e–h*) which were undetectable in the patterns of normal high-sulfur proteins. We feel that there are many other components falling into this category, particularly in TTD fraction IV, but which we cannot unequivocally identify. Although TTD high-sulfur protein contained a few (about 6) constituents with mobilities corresponding to normal components, there were at least 8 major normal components, marked with arrowheads on Fig 6*a–d* which could not be detected in TTD protein patterns.

Two areas of the two-dimensional patterns provided special problems in interpretation. The wedge of UHS proteins in normal fraction I (Fig 6*a*) seriously reduced the clarity of underlying spots and it was not possible to make an adequate count of normal high-sulfur proteins in this major fraction. However for the purpose of making comparisons, the consequences were minimal for few of the proteins in TTD fraction I run in this area of the electrophoretogram. There were many poorly resolved low M_r components visible in Fig 6*d,h,* and it is not possible to make adequate comparisons between the normal and abnormal components because of the absence of identifiable reference components. We can conclude that the two protein fractions differ in at least 30 resolvable

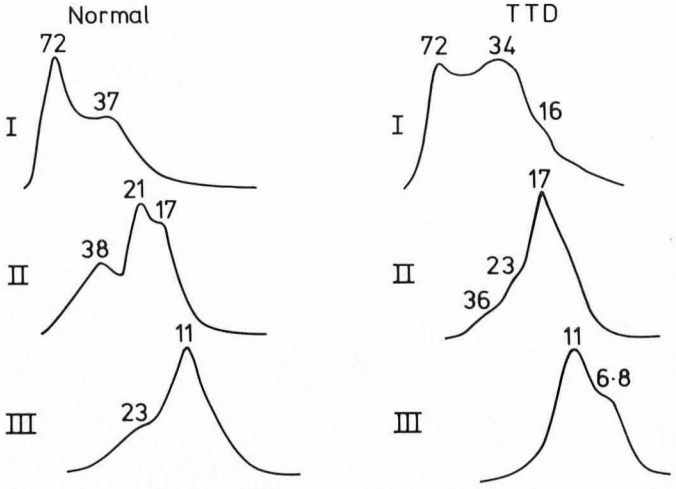

FIG 4. Elution profiles obtained by HPLC in 0.1 M phosphate–8 M urea at pH 7 of hair high-sulfur protein fractions obtained by chromatography on Sephadex G-200 (see Fig 3). The column was calibrated with respect to M_r with 3 wool high-sulfur proteins of known sequence M_r. Peak tube M_r are given in kilodaltons.

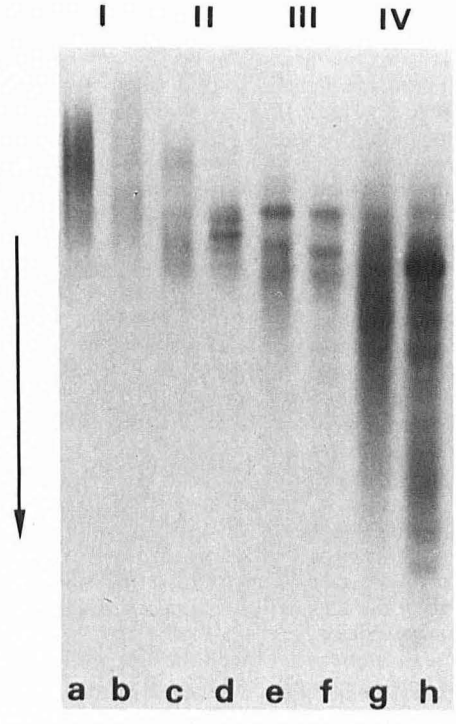

FIG 5. Electrophoretic patterns of high-sulfur protein fractions obtained by chromatography on Sephadex G-200 (see Fig 3). *a, c, e, and g,* Normal hair fractions I–IV. *b, d, f, and h,* TTD hair fractions I–IV. Run in 10% PAA gels containing 3 M urea at pH 2.6. The track corresponding to TTD I was underloaded.

components together with an unknown number of UHS proteins. These Sephadex fractions, except TTD fraction IV and normal fraction V for which there was lack of material, were also compared by amino acid analysis (Table II), giving 3 pairs of corresponding analyses and analyses of normal IV and TTD V. It can be seen (Table II) that corresponding fractions are quite different in composition, generally reflecting the differences found between the parent unfractionated high-sulfur proteins with the TTD fraction of each pair containing substantially less SCMC but more aspartic acid, glycine, alanine, leucine, and isoleucine than the corresponding normal fraction. Such differences in composition also observed between the

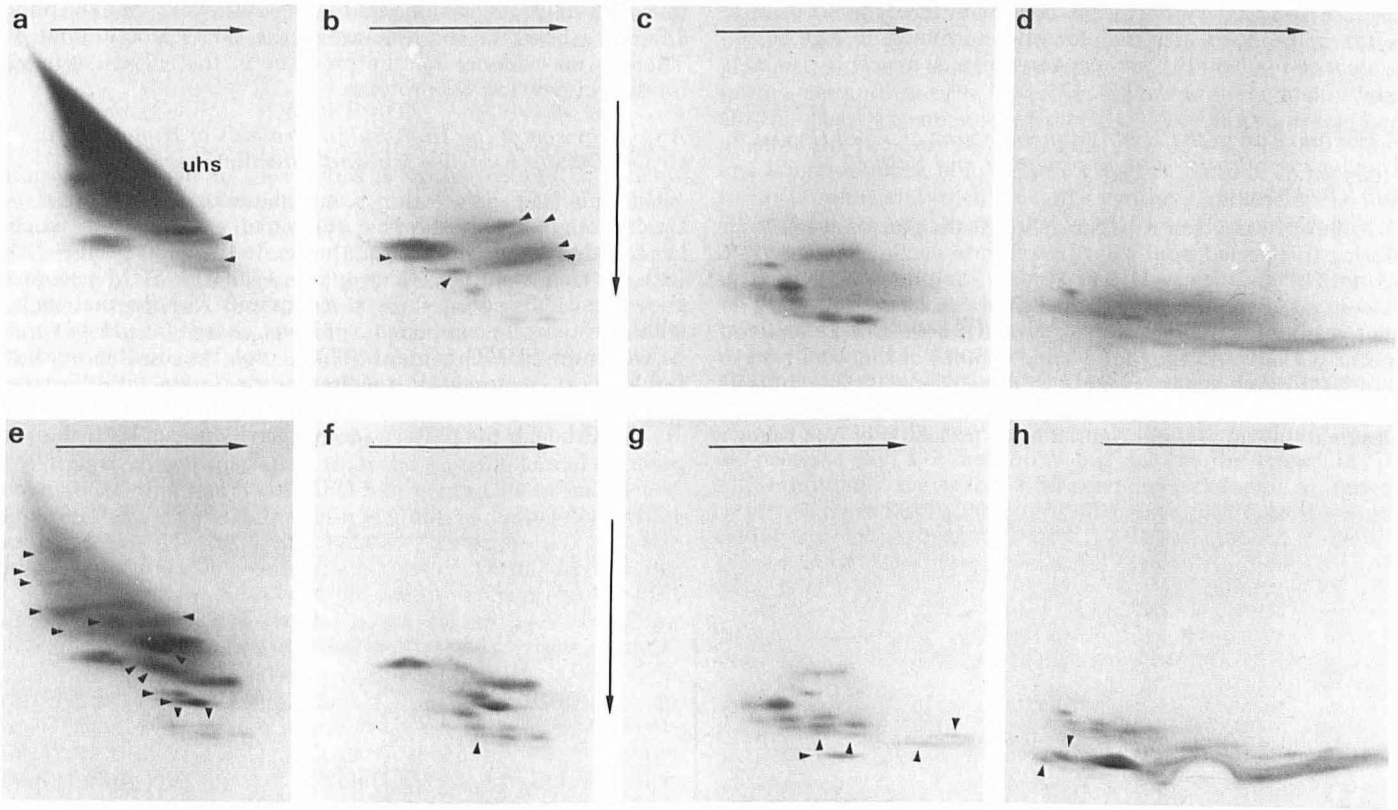

FIG 6. Two-dimensional electrophoretic patterns of high-sulfur protein fractions obtained by chromatography on Sephadex G-200 (see Fig 3). *a–d,* Normal hair fractions I–IV. *Arrows* indicate those components which are detectable only in this hair. *e–h,* TTD hair fractions I–IV. Arrows indicate those components which are detectable only in TTD hair. In each set components are marked only once although they may appear in more than one fraction. For gels *a–c* and *e–g* the origins were located approximately in the top left hand corners of the photographs. For gels *d* and *h,* in order to allow visualization of the faster-moving components in the first dimension, part of the gel photograph has been cut off and the point of application of the protein would be somewhat to the left of the edge. First dimension (horizontal), electrophoresis in 10% PAA gels containing 3 M urea at pH 2.6. Second dimension (vertical), electrophoresis in continuous gradient PAA gels containing SDS at pH 7.

TABLE II. *Amino acid compositions (as residues %) of high-sulfur protein fractions isolated from normal and TTD hair (see Fig 3)*

	Sephadex fraction no.							
	I		II		III		IV	V
	Normal	TTD	Normal	TTD	Normal	TTD	Normal	TTD[a]
Lysine	0.5	1.2	0.5	0.7	0.6	0.9	0.6	2.1
Histidine	0.9	0.5	0.9	0.6	1.1	0.8	1.0	0.9
Arginine	6.6	3.9	6.3	5.4	6.3	5.4	6.2	4.5
SCMC[b]	28.9	21.1	27.1	18.8	25.5	16.9	24.7	12.7
Aspartic acid	1.8	2.3	2.0	3.1	2.8	4.3	2.5	6.0
Threonine	10.8	7.3	11.1	10.1	10.8	9.9	10.7	6.3
Serine	13.4	16.3	13.2	13.7	12.1	13.0	13.3	14.8
Glutamic acid	8.6	9.6	8.6	8.8	7.6	7.8	6.9	8.8
Proline	11.8	12.5	12.5	11.6	13.0	11.2	12.2	9.5
Glycine	4.7	5.6	5.5	7.7	5.4	8.0	6.6	12.6
Alanine	1.7	4.8	1.9	3.2	2.0	3.6	2.7	5.1
Valine	4.9	7.8	4.6	7.3	5.0	7.3	5.0	4.5
Methionine	0.0	0.3	0.0	0.3	0.2	0.3	0.2	tr[c]
Isoleucine	1.4	1.7	1.4	2.3	1.8	2.7	1.4	2.2
Leucine	1.7	2.8	2.0	3.3	3.3	4.6	2.8	4.8
Tyrosine	1.5	1.5	1.5	1.7	1.4	1.6	1.6	2.5
Phenylalanine	0.9	1.0	1.0	1.4	1.2	1.8	1.4	2.7

[a] This fraction also contained small amounts of ornithine and citrulline.
[b] S-carboxymethylcysteine
[c] tr – trace

hairs (Table I) therefore stem not only from a loss by the TTD hair of the UHS proteins but also from a general shift in synthesis to high-sulfur protein components of lower cystine content than normal. In the present study we found that at least 96% of normal high-sulfur protein contained 25 residues % or more of SCMC, while Pollitt and Stonier [6] using a different fractionation procedure found that virtually all their normal high-sulfur fractions contained 20 residues % or more of this amino acid. It is therefore of considerable interest to note that apart from fraction I, all TTD high-sulfur fractions were below 20 residues % of SCMC. On this basis alone it would therefore appear that at least 85% of the TTD high-sulfur proteins have lower SCMC content than components normally present in hair and thus must be classed as abnormal. TTD

fraction V has a very low SCMC content of 12 residues %, which is far below that recorded for any normal hair high-sulfur protein component [6] but appears similar to a recently isolated minor component of wool [17].

A Comparison of the High-Sulfur Proteins of Wool Grown During Periods of Sulfur-Deprivation and Sulfur-Enrichment

A sheep was given a sulfur-deficient diet for 12 weeks and during this period wool mass growth rate declined to only 11% of normal and the wool half-cystine content, already at the lower end of the range, remained at about 9.9 residues %. The diet was then supplemented for 6 weeks with 1.34 g elemental sulfur per day and the half-cystine content of the wool rose to 11.2% [18]. An examination of two-dimensional electrophoresis patterns of the high-sulfur proteins produced during the sulfur-deprivation and the sulfur-enrichment periods (Fig 7*a, b*) shows that both fractions give rise to the same array of spots, the only difference being in the amount of the UHS protein wedge. There is no evidence for the presence in the sulfur-deprived fraction of new low M_r proteins.

A Comparison of the High-Sulfur Proteins of Human Hair Grown During Kwashiorkor and After Recovery

Gillespie [19] showed that hair high-sulfur proteins synthesized during the kwashiorkor state had on average a much lower half-cystine content than normal (19 vs. 30 residues %) with moving boundary electrophoresis of the SCM proteins showing a substantial shift, as compared with normal high-sulfur proteins, to components of lower charge (at pH 4.5) and hence lower SCMC content. These proteins, now somewhat insoluble after storage in the freeze-dried state for 16 years, have been compared by two-dimensional electrophoresis (Fig 7*c, d*). Although the patterns are rather indistinct both show a

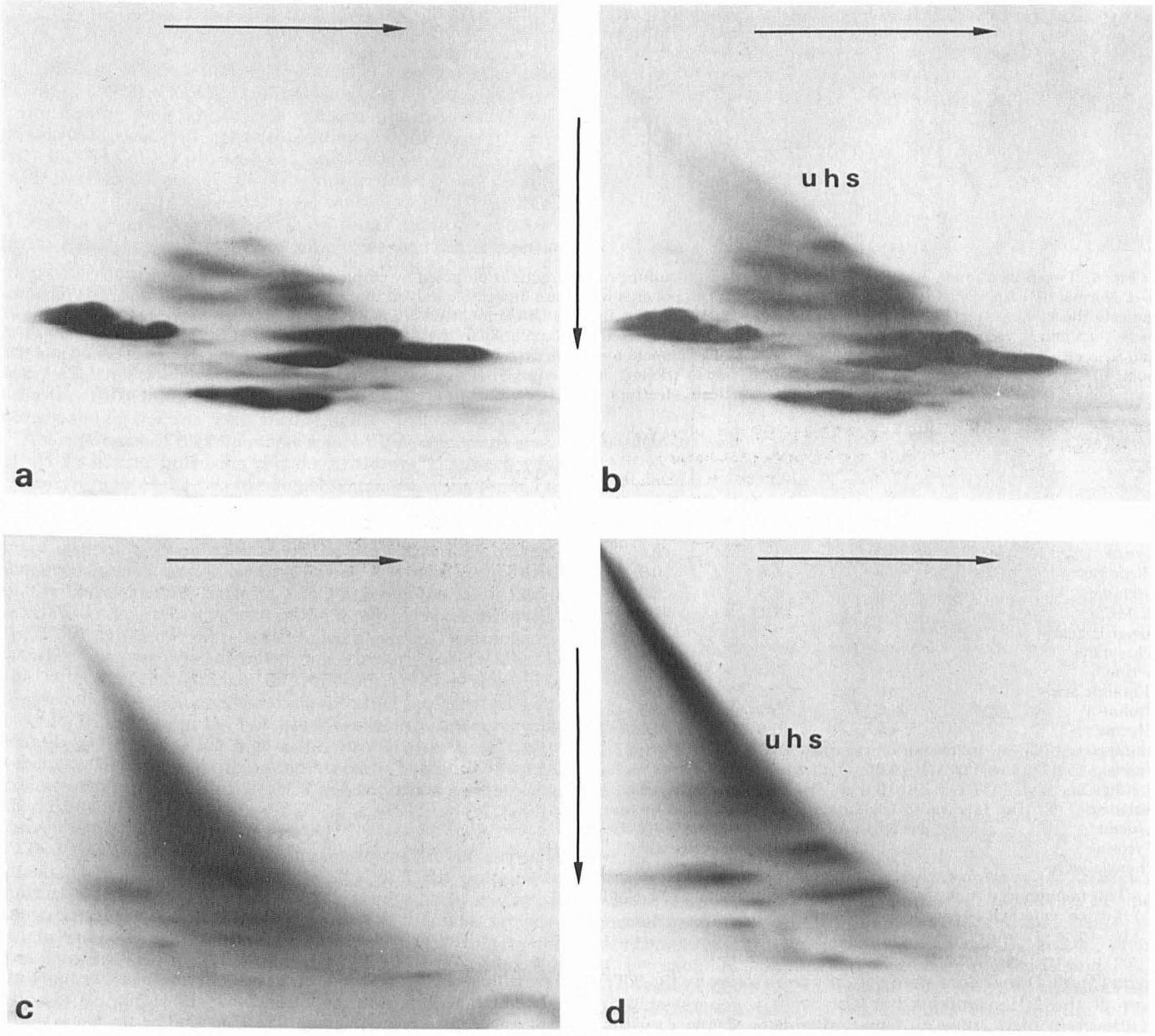

FIG 7. Two-dimensional electrophoretic patterns of high-sulfur proteins of wool (*a,b*) and hair (*c,d*) produced under different dietary conditions. *a*, Wool grown during sulfur-deficiency. *b*, Wool grown during sulfur enrichment. *c*, Human hair grown during kwashiorkor. *d*, Human hair grown after recovery. First dimension (horizontal), electrophoresis in 10% PAA gels containing 3 M urea at pH 2.6. Second dimension (vertical), electrophoresis in continuous gradient PAA gels containing SDS at pH 7.

similar array of spots with the kwashiorkor pattern (Fig 7*c*), showing less of the UHS fraction but a significant amount of low M_r protein.

DISCUSSION

The sample of hair examined in this study meets the definition of TTD hair in that it has a cystine content far below normal (< 50% of normal) with a grossly abnormal amino acid composition. Similar materials have been studied by Pollitt and Stonier [6], Gold and Kachra [20], and Baden et al [21]. Such a change in the composition of hair obviously reflects changes in the constituent proteins. We can visualize this taking a variety of forms: a simple change in the overall proportions of the low-sulfur and high-sulfur proteins, an alteration in the relative proportions of their subunit polypeptides, or the appearance of new protein components. The present situation may provide examples of all three.

We have shown that apart from the possible loss of a minor component, this sample of TTD hair contains a normal set of low-sulfur protein subunit polypeptides covering the usual range of M_rs and isoelectric points. These data rule out the possibility of the mutation having affected the size of the polypeptides or the relative proportions of constituent charged amino acids, to which can be added cystine because of the presence of its charged derivative S-carboxymethylcysteine. The two-dimensional electrophoretic technique described in this paper is an ideal tool for looking for mutational changes in these proteins and there is an urgent need for its application to other samples of TTD hair particularly those (e.g., sample IV-6 of Baden [4] and that of Pollitt and Stonier [6]) for which there is some evidence for the presence of mutated low-sulfur proteins.

In contrast, the high-sulfur proteins are greatly decreased in amount and those present have a lower than normal average M_r and cystine content. These changes in the nature of the high-sulfur proteins are due to 4 well-defined events involving the TTD hair: (1) The loss of the heterogeneous group of UHS proteins. (2) The loss of at least 8 high-sulfur proteins that figure prominently in the electrophoretic pattern of normal hair high-sulfur protein. (3) The acquisition of at least 22 high-sulfur proteins of lower than normal cystine content that are undetectable in normal hair. (4) The acquisition of a significant amount of abnormal ill-defined low M_r protein.

Event 1, the loss of the UHS proteins, an effect common to sulfur deprivation in the sheep and protein deficiency in humans, provides strong evidence supporting the hypothesis that TTD hair is grown under conditions of sulfur deficiency. As the subject was on an adequate diet this deficiency must be a metabolically induced sulfur deficiency at the follicle and may involve inter alia, changes in the absorption, transfer, or metabolism of sulfur-containing amino acids. However this may not be the cause of the other events, for sulfur deprivation in the sheep, carried to the stage where wool growth almost ceased, does not cause the loss of normal proteins, apart from the loss of the UHS proteins, or the appearance of new ones. In fact over a long series of experiments involving the dietary, hormonal, and physiologic manipulation of the sheep, the only changes observed in wool high-sulfur proteins have involved the UHS fraction [22]. This appears to be true also for the high-sulfur proteins in kwashiorkor, although this hair does appear to contain some very heterogeneous low M_r material. These results suggest that events 2–4, i.e., the loss of normal high-sulfur proteins and the acquisition of many "new" proteins, are not the result of sulfur deficiency but stem from the effects of a different regulatory mechanism. In interpreting these changes it is unfortunate that we do not have sufficient information to decide whether the 22 "new" proteins are new in the sense of being mutated or whether they belong to the normal spectrum of high-sulfur proteins but are produced by the follicle of normal hair in undetectable amounts. The fact that there is the same inverse correlation between SCMC content and the content of aspartic acid, glycine, alanine, valine, isoleucine, leucine, and phenylalanine previously found for wool high-sulfur proteins [23] suggests that these "new" proteins are in fact part of a normal set and that the latter hypothesis is a reasonable one. On this basis we can suggest that the TTD mutation has not affected the proteins per se but has altered a regulatory gene, one effect of which is to suppress the synthesis of some high-sulfur proteins and to enhance the synthesis of others, the net effect being the appearance of a virtually new set of high-sulfur proteins.

An alternative hypothesis suggests that a set of apparently normal high-sulfur proteins is synthesized in the follicle but then partly degraded, perhaps by the lysosomal enzymes released during the latter stages of fiber formation [24]. This attack could result from changes in the nature of the enzymes, in the proteins, or in the millieu of the cell. Such a degradative process may well be responsible for the production of the low M_r proteins of TTD fraction V but not for the "new" high-sulfur proteins because the latter span much the same M_r range as those of normal hair in spite of their very different amino acid composition. Nevertheless this theory should be taken into consideration in future studies of TTD hair.

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A Comparative Study of the Physical and Chemical Properties of Melanins Isolated from Human Black and Red Hair

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Some of the physical and chemical properties of the melanins isolated from human black and red hair were compared. Some of these properties were also compared with those of synthetic dopa melanin. Five samples each of black and red hair were used for isolating melanin by extraction with 2.5 M NaOH, and purification by repeated precipitation with HCl and redissolution in NaOH. The proteins associated with the melanins were hydrolyzed by refluxing the melanoproteins in 6 M HCl. The melanoproteins from black and red hair had 55.8 ± 2.0% and 41.5 ± 2.5% melanin, respectively. There was no statistically significant difference between the amino acid compositions of the hydrolysates from black and red hair melanoproteins. The ultraviolet and visible spectra of red hair melanin showed significant differences from those of black hair melanin and dopa melanin. Even though the overall spectra of red hair melanin showed some differences from those of black hair and dopa melanins, the results indicate a close similarity in the groups identifiable by the IR spectra. The compositions of C, H, N, S, and O in these melanins were determined. Red hair melanin contained more S than black hair melanin; dopa melanin did not contain any S. Black and red hair melanins oxidized NADH at approximately the same rate. Ultraviolet irradiation increased the oxidation of NADH by red hair melanin to a greater extent than that by black hair melanin. These results show that although there are general similarities, there are significant differences in the physical and chemical properties of melanins isolated from black and red hair.

Clinical experience has shown that there is a higher incidence of skin cancer and increased photosensitivity in people with red hair and light skin compared to those with dark hair and skin. This is generally attributed to the lower amounts of melanin present in the skin of the former group and/or differences in the type of melanin in these individuals. It is widely accepted that light colored hair has predominantly pheomelanin, in contrast to the eumelanin usually present in dark hair [1–3]. Eumelanin is chemically different from pheomelanin, the most obvious difference being the higher sulfur content of pheomelanin compared to eumelanin. It seems that there are two separate, but possibly interrelated, pathways for the biosynthesis of these two types of melanins. Eumelanin is synthesized by the oxidation of dihydroxyphenylalanine (dopa) to dopaquinone and a series of subsequent oxidation and polymerization reactions. On the other hand the synthesis of pheomelanin proceeds via the condensation of dopaquinone with cysteine to produce cysteinyl dopa and the oxidation and polymerization of cysteinyl dopa [1–6].

Chedekel and associates have shown that pheomelanin is degraded by irradiation with long-wavelength ultraviolet (UV) and visible light [7–10]. It has also been observed that under these conditions superoxide is formed. We have recently conducted a comparative study of the formation of superoxide during irradiation of eumelanin and pheomelanin (unpublished data).

A number of reports have demonstrated that several eumelanins, synthesized from different chemicals as well as those isolated from melanoma and human black hair, participate in oxidizing and reducing reactions [11–13]. The oxidation of NADH by dopa melanin has been found to be increased by irradiation with visible light [13,14]. Comparative data on the effects of UV or visible radiation upon the NADH oxidation by pheomelanin is included in this paper.

The eumelanins are known to exhibit specific electron spin resonance (ESR) signals; these signals are enhanced by visible irradiation [15–17]. Recent comparisons of the ESR signals of eumelanin and pheomelanin have shown that there are significant differences between the signals of eumelanins and pheomelanins [18–20].

This paper describes a comparison of some of the physical and chemical properties of melanins isolated from human black

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Abbreviations:
ESR: electron spin resonance